Pulmonary Metastases of Breast Carcinomas: Ligandohistochemical, Nuclear, and Structural Analysis of Primary and Metastatic Tumors With Emphasis on Period of Occurrence of Metastases and Survival

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Background and Objectives: Pulmonary metastases of breast carcinomas have a high frequency and are often subject to surgical intervention. To contribute to advances in the knowledge about morphometric and biochemical parameters of primary tumors and their metastatic lesions, analysis of syntactic structure and thermodymanic aspects as well as of expression of distinct glycohistochemical features with respect to period of metastasis occurrence and patient survival is desirable.
Methods: Clinical history, surgical findings, histopathological reports, survival of the patients with a maximum follow-up of 15 years, and paraffin blocks of 32 breast carcinoma specimens and their pulmonary metastases were examined. Only potentially curative resections of both the breast carcinoma and their metastases have been included for anlysis. The following markers were applied: neoglycoconjugates with histoblood group A- and H-trisaccharides, lactose, α-N-acetyl-D-galactosamine and the Forssman disaccharide, a polyclonal immunoglobulin G fraction from human serum with specificity for 9-0-acetylated sialic acid, which is a tumor marker for melanomas, the serum lectins serum amyloid P component and mannan-binding lectin, the mannose-specific plant lectin concanavalin A, and monoclonal antibodies specific for estrogen and progesterone receptors, respectively. In addition, measurements of the integrated optical density (IOD) and tissue structure were performed.
Results: The frequency of expression of hormone receptors and expression of binding capacities to most of the applied probes was similar between the primary and metastatic tumors; however, it varied markedly between different patients. For the IOD parameters, a close association between the primary tumors and their metastases was seen, especially a rather low S-phase-related tumor cell fraction and a high percentage of

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tumor cells with an IOD >5C. The mean time for the development of intrapulmonary metastases measured 43 months. It was considerably longer in progesterone receptor-negative tumors (49.7 months) and those with a lack of expression of sites with specificity for the Forssman disaccharide (48.7 months). The survival was positively correlated with the presence of binding capacity of histoblood group A-trisaccharide and certain structural parameters, especially the structural entropy and its current. The presence of estrogen and progesterone receptors was not associated with the total survival at a statistically significant level.

Conclusions: Histochemical features between the primary breast carcinoma and their intrapulmonary metastases can evidently vary. Analysis of the hormone receptor status in metastatic lesions seems to be useful for diagnostic purposes only in rare cases, i.e., distinguishing metastases from primary lung carcinoma. Nonetheless, the survival of patients with metastasizing breast carcinoma is associated with features of the primary tumors, especially the detection of binding capacities for the Forssman disaccharide and the histoblood group A-trisaccharide. Extent of lymph node involvement of the breast carcinoma is not prognostic for later pulmonary involvement. *J. Surg. Oncol. 1998;69:137–146.*

KEY WORDS: breast neoplasms; lung metastases; lectins; survival; histoblood group trisaccharides

INTRODUCTION

Metastatic lesions in the lungs from various tumor types are commonly encountered, and surgical treatment of pulmonary metastases is an important task in adjuvant tumor surgery [1,2]. Although surgical excision of metastases will not be able to definitely cure the patient, survival rates of patients with resected intrapulmonary metastases are encouraging and comparable to those of patients with primary lung carcinoma [1,2]. The survival of patients with intrapulmonary metastases appears to be negatively affected by the number of metastatic lung lesions and positively by the period between the resection of the primary tumor and that of lung metastases [1,2]. Within the group of patients with lung metastases, women with primary breast carcinoma contribute a sizable fraction. In fact, development of lung metastases has been reported to occur in 10–20% of all women with breast carcinoma [3,4]. Primary breast carcinomas exhibit distinct features that are not frequently found in other malignancies: they are euploid tumors to a high percentage, display a low proliferation rate as measured by Ki67 immunostaining or DNA cytometry, and the median survival of affected women amounts to 5–8 years and is longer than that of other tumor types such as lung or stomach carcinomas [5–8]. Except for the clinical stages, the post-surgical TNM stages, and the hormone receptor status, no reliable factor has been reported to meet general approval which permits an estimation of the patient's prognosis to a satisfying degree [3,5,9–11]. The proliferation or mitotic index and molecular biological parameters such as the expression of c-erb, p53, NCAM, and related molecules, of the cytometric DNA characterization are currently considered to warrant further examination prior to a conclusive statement.

Following this line of reasoning, the assessment of the period between detection and excision of the primary breast carcinoma and the lung metastases, the tumor features defined by the post-surgical TNM stage, and the overall survival of the patients were the main objectives of this study. In addition, cytometric measurements and syntactic structure analysis were performed to compare the respective results between the primary breast carcinoma and their lung metastases. Analysis of expression of receptors that have been reported to harbor prognostic significance, i.e., estrogen and progesterone receptors, is included. Emphasis is also placed on the monitoring of the capacity of the tumor cells to interact with the carbohydrate part of cellular glycoconjugates. Owing to the information-storing potential of sugar structures, the recognitive interplay between carbohydrates and suitable receptors such as lectins is assumed to guide and regulate diverse cellular activities including cell adhesion and proliferation [12–15]. Since endogenous lectins have already been detected immunohistochemically in primary breast cancer [16], further monitoring of potentially relevant activities can be pursued by reverse lectin histochemistry with the carrier-immobilized carbohydrate ligand [17,18]. In addition to the histoblood group A- and H-trisaccharides, which have indicated a prognostic value for receptor presence in lung cancer [19], we have focused on receptor expression for N-acetyl-D-galactosamine as single unit or as Forssman disaccha-

ride (galNAc-α1,3-galNAc-β1R). This selection was prompted by the results in model systems or tissue sections obtained with plant lectins or neoglycoenzymes that the occurrence of this sugar or respective receptors appears to be linked to metastatic capacity [20–26]. Tumor cells can encounter molecules of the innate immune system, i.e., collections or pentraxins, during their passage from the primary site to the new target environment which could translate into a selection process [12,27]. Thus we also employed a member of each group of proteins as glycohistochemical tools. We chose the collectin mannan-binding lectin (MBL) and the pentraxin serum amyloid P (SAP) component. A similar selection can be operative with respect to naturally occurring immunoglobulin fractions, among them one with selectivity to 9-0-acetylated sialic acid (Neu5,9-Ac_2) [28,29]. Due to the suggested relevance of concanavalin A (Con A) reactivity for metastasis formation and survival from a cell model and histochemical analysis [26,30,31], this plant lectin was also part of the glycohistochemical panel of this study. Binding of this lectin was compared with that of the collectin to infer differences of the two lectins with identical monosaccharide specificity. This result has relevance for the interpretation of application of plant lectins which are not simply mimicking the properties of endogenous lectins.

MATERIALS AND METHODS

Patients and Clinical Data

From 32 women who underwent potentially curative resections of their breast carcinoma and lung metastases clinical data and paraffin-embedded specimens of the primary and the lung metastases were available. Information on survival times was obtained by response to questionnaires repeatedly sent to the house physicians. The maximum follow-up from the excision of the breat carcinoma was 170 months and from the resection of lung metastases was 100 months.

Histology and Image Analysis

The tissue blocks were cut in 4–5 μm sections and processed with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS), and Feulgen stains for tumor classification as well as cytometric and syntactic structure analysis. The number of stem lines, the percentage of tumor cells with an integrated optical density (IOD) >3C, or >5C, the S-phase-related tumor cell fraction, the entropy [according to 32–34], and the current of entropy of the metastases were measured on Feulgen-stained slides by an automated image analyzing system. The self-written programs were based upon the commercially available DIAS language (Towersoft, Berlin, Germany) and also include syntactic structure analysis of the two-dimensional images. Briefly, the centers of tumor cell and lymphocyte nuclei were computed, and the corresponding X-Y coordinates served for the computation of the path-(tree-)-like network with the shortest distances between the nuclear centers, the so-called minimum spanning tree (MST). This network can be based upon all cells or certain cellular subfractions such as tumor cells, lymphocytes, or cells displaying a comparable nuclear feature (S-phase-related IOD, IOD >5C), etc. The construction of the MST readily enables the assessment of distances between neighboring tumor cells, tumor cells and lymphocytes, proliferating tumor cells, tumor cells with an IOD >5C, and of the MST entropy and the current of MST entropy (lung metastases only) [32].

Immuno- and Ligandohistochemistry

The presence of estrogen and progesterone receptors was visualized immunohistochemically with monoclonal antibodies obtained from Biotrend (Cologne, Germany). Immunohistochemical detection of hormone receptors included a pretreatment of the sections with microwaves (3 × 5 min at 600 W in citric acid) and was performed as prescribed by the protocol of the probe distributor (Biotrend). Glycohistochemical analysis included the use of two endogenous lectins (the collectin MBL and the pentraxin SAP component), the mannose-specific plant lectin Con A, and a Neu5,9-Ac_2-specific immunoglobulin G fraction from human serum. In addition, a panel of biotinylated neoglycoconjugates, i.e., lactosylated and α-N-acetylgalactosaminylated bovine serum albumin and substituted poly(2-hydroxyethylacrylamide) with histoblood group A- and H-trisaccharides and the Forssman disaccharide, respectively, as ligand part was tested systematically. Their synthetic preparation or purification, labeling under activity-preserving conditions, and the set of positive and negative controls were performed, as described in detail elsewhere [18,19,27–29,35,36]. The ligandohistochemical procedures were based upon the avidin-biotin complex (ABC) technique, using a concentration of 10 μg/ml and an incubation time of 60 min, as described previously [32,37,38]. In both techniques, the conventional peroxidase-antiperoxidase method was used with horseradish peroxidase and diaminobenzidine/H_2O_2 for visualization of the specifically bound probes. Cases were classified as positive if a dark-brown color was seen in all or in clusters of the tumor cells.

Statistical Analysis

All statistical tests were performed by use of the commercially available program package NCSS for Windows (Number Cruncher Statistical Systems, Kaysville, UT). The survival rates were computed according to the common Kaplan-Meier method. The statistical tests included the log-rank test for survival analysis, chi-squared test, Fisher's exact test, and non-hierarchic multivariant

TABLE I. Synopsis of Clinical Parameters in Patients With Primary Breast Carcinoma (N = 32) and Subsequent Intrapulmonary Metastases

Parameter	Primary tumor	Lung metastasis
Mean age (years)	50.5 ± 11.1	54 ± 10.6
pT stages		
pT1	10	
pT2	19	
pT3	2	
pT4	1	
Tumor diameter (cm)	2.8 ± 1.7	2.8 ± 2.9
pN stages		
pN0	19	
pN1	10	
pN2	1	
pN3	2	
Median survival (months)	59	40[a] (20[b])

[a]Median period (excision of primary breast carcinoma to resection of lung metastasis).
[b]Median survival (resection of lung metastasis to death).

discriminant analysis. *P* was regarded as significant at the level of <0.05.

RESULTS

The clinical information on the 32 patients including pT and pN stages, the median periods from the excision of the breast carcinoma until the resection of lung metastases, and the median survival of patients was collected (Table I). The mean diameter of the breast carcinomas measured 2.8 cm. Occurrence of advanced tumor stages was rather infrequent. The median survival of the operated women was 59 months. Intrapulmonary metastases were excised after a median period of 40 months. Since material from primary and secondary lesions of the same patient was available, it was possible to compare various features between sections of each primary breast tumor and the corresponding lung metastases originating from this tumor. As compiled in Tables II and III, the results for each cyto- and histometric variable are presented with respect to positivity of immuno- and glychohistochemical parameters. The presence of binding capacities for the histoblood group trisaccharides was associated with a low S-phase-related tumor cell fraction and a low current of structural entropy in the primary breast carcinomas and metastases, primarily for the H-trisaccharide. This observation is in accordance with data reported from lung carcinoma [19]. This report extends the observation also to the Forssman disaccharide, whereas the simple monosaccharide N-acetyl-D-galactosamine as ligand part was less discriminatory. Among the hormone receptors, positive estrogen receptor status yielded a rather similar correlation (Table III). Due to the uneven distribution in the positive/negative groups for the probes from the innate and adaptive host defense system, the statistical differences should be viewed with caution (Table III). When the lung metastases were likewise analyzed, a marked reduction in the number of galNAc- and A-trisaccharide-binding cases and an increase in the number of H-trisaccharide-binding cases were observed (Table III). The entropy flow could even exceed the level of the receptor-negative cases for the probes localizing estrogen receptor and A-/H-trisaccharide-binding sites (Table III). The association of presence of distinct binding properties with this thermodynamic characteristic is apparently different in primary and secondary lesions (Tables II, III). The two lectins with identical specificity in D-mannose gave a different extent of positivity in this group of patients.

The relationship between the histochemical detection of receptors in breast carcinoma and their lung metastases and the associated periods (median survival times calculated from the excision of the breast carcinoma and of their lung metastases, respectively) is depicted in Table IV. The reactivity to A-trisaccharide clearly correlated with prolonged periods (overall survival, period until excision of metastases, and survival after metastases resection). The reactivity of the cells to the Forssman disaccharide, which has the terminal α-GalNAc moiety in common with A-trisaccharide, reflects the opposite trend (Table IV). The expression of estrogen and progesterone receptors in the breast carcinomas is associated with rather fast development of metastases. However, a prolonged survival of patients after resection of the intrapulmonary metastases can be discerned. By computation it is tested whether the level of the IOD entropy can estimate the period of development of lung metastases (Fig. 1) and whether the distance between tumor cells and lymphocytes (Fig. 2) and the current of structural entropy provide indications for the length of the overall survival (not shown). The patients have a favorable prognosis if the distance between tumor cells and lymphocytes is rather small and the current of structural entropy is high. Of similar potential importance for the survival of patients, the period between excision of the primary breast carcinoma and their pulmonary metastases and the survival after resection of lung metastases apparently is the analysis of expression of binding capacities for the A-trisaccharide (Figs. 3–5) and for the Forssman disaccharide (not shown). Even within the small cohort of 32 patients a clear separation of the survival curves can be noted at a highly statistically significant level ($P < 0.01$).

The results of the multivariate analysis of the most significant features associated with the overall survival, length of period until lung metastasis resection, and survival after excision of metastases are summarized in Table V. The age of the patients and expression of binding capacities for the synthetic histoblood group A-trisaccharide as well as the two noted cytometric and histometric features are correlated with the overall survival and time of metastasis resection. For the survival

TABLE II. Relationship Between Cytometric and Histometric Features and Expression of Glycohistochemical Binding Capacities in Primary Breast Carcinoma (Np = 32)*

Probe	Np	S-phase-related fraction	% >5C	IOD Entropy	IOD Entropy flow	Distance between tumor cells and lymphocytes	MST Entropy	MST Entropy flow
A-tri$^+$	25	9.4	45.2	1.7	5.8	55	18	42
A-tri$^-$	7	10.3	49.9	1.7	6.8	55	18	52
GalNAc$^+$	29	10.2	45.3	1.7	6.3	53	18	46
GalNAc$^-$	3	9.0	55.7	1.7	3.0	69	20	32
Lac$^+$	17	9.3	51.2	1.6	5.3	55	19	40
Lac$^-$	15	11.0	40.7	1.7	6.8	55	17	50
H-tri$^+$	12	7.6	56.4	1.6	4.7	51	20	42
H-tri$^-$	20	11.6	40.2	1.7	6.8	57	17	46
FS-di$^+$	10	6.5	62.8	1.5	3.5	56	22	38
FS-di$^-$	22	11.7	38.7	1.7	7.2	54	16	48
IgG$^+$	20	9.8	46.2	1.7	6.3	55	19	45
IgG$^-$	12	10.5	46.3	1.6	5.6	58	17	44
Prog$^+$	18	10.7	44.1	1.7	5.2	57	19	38
Prog$^-$	14	9.4	49.1	1.6	7.1	55	17	52
Estr$^+$	19	8.8	51.6	1.7	5.0	59	20	40
Estr$^-$	13	11.9	38.4	1.7	7.6	52	15	51
SAP$^+$	6	8.3	59.7	1.4	4.0	61	21	37
SAP$^-$	26	10.5	43.2	1.8	6.5	55	17	46
MBL$^+$	26	9.8	48.2	1.7	5.0	57	19	38
MBL$^-$	6	11.5	37.8	1.7	10.6	52	16	72
Con A$^+$	30	9.7	47.1	1.7	4.9	57	18	37
Con A$^-$	2	15.5	34.0	1.7	22.5	45	15	160

*Np = number of cases with primary breast carcinoma; A-tri = histoblood group A-trisaccharide; GalNAc = α-N-acetyl-D-galactosamine; Lac = Lactose; H-tri = histoblood group H-trisaccharide; FS-di = Forssman disaccharide; IgG = Anti-Neu5,9-Ac_2 antibody; Prog = progesterone receptor; Estr = estrogen receptor; SAP = serum amyloid P component; MBL = mannan-binding lectin; Con A = concanavalin A.

TABLE III. Relationship Between Cytometric and Histometric Features and Expression of Glycohistochemical Binding Capacities in Lung Metastases of Breast Carcinoma (Nm = 32)*

Probe	Nm	S-phase-related fraction	% >5C	IOD Entropy	IOD Entropy flow	Distance between tumor cells and lymphocytes	MST Entropy	MST Entropy flow
A-tri$^+$	20	9.0	51.7	1.6	7.2	49	21	61
A-tri$^-$	12	13.8	31.8	1.8	4.9	50	13	27
GalNAc$^+$	15	9.9	49.3	1.6	4.8	51	20	42
GalNAc$^-$	17	11.5	39.7	1.8	7.7	48	16	54
Lac$^+$	18	10.1	46.7	1.6	5.8	49	19	49
Lac$^-$	14	11.7	41.0	1.7	7.0	41	17	48
H-tri$^+$	17	9.2	49.7	1.6	6.9	49	18	55
H-tri$^-$	15	12.6	38.0	1.7	5.6	51	18	41
FS-di$^+$	10	10.8	44.1	1.7	6.9	48	17	46
FS-di$^-$	22	10.8	44.3	1.7	6.0	50	19	49
IgG$^+$	22	10.0	47.4	1.6	7.0	48	20	54
IgG$^-$	10	12.5	37.2	1.8	4.8	52	14	35
Prog$^+$	18	9.1	51.2	1.6	6.4	48	21	54
Prog$^-$	14	12.9	35.2	1.8	6.2	51	14	41
Estr$^+$	18	10.2	47.1	1.7	7.0	49	19	53
Estr$^-$	14	11.6	40.5	1.6	5.4	50	17	42
SAP$^+$	8	9.8	51.1	1.7	8.1	49	18	68
SAP$^-$	24	11.1	41.9	1.7	5.7	50	18	42
MBL$^+$	26	10.4	46.1	1.7	6.2	49	19	52
MBL$^-$	6	12.7	36.2	1.5	6.7	53	15	32
Con A$^+$	31	10.7	44.5	1.7	6.5	49	18	50
Con A$^-$	1	113.0	35.0	1.8	5.7	69	13	48

*Nm = number of cases with pulmonary metastases; for further abbreviations, see footnote to Table II.

TABLE IV. Median Period (Months) Between Resection of Breast Carcinoma and Lung Metastasis and Median Survival (Months) of Breast Carcinoma Patients in Relation to Expression of Receptor Activities of Breast Carcinoma and Median Survival After Resection of Lung Metastases (Months) in Relation to Expression of Receptor Activities in Lung Metastases (N = 32)*

Probe	Np	Median period for occurrence of lung metastases	Median survival after excision of breast carcinoma	Nm	Median survival after resection of lung metastases
A-tri^{+}	25	43.6	66.0	20	22.3
A-tri^{-}	7	28.4	49.7	12	8.1
GalNAc^{+}	29	40.6	57.9	15	20.3
GalNAc^{-}	3	28.4	50.7	17	22.3
Lac^{+}	17	32.5	58.8	18	22.3
Lac^{-}	15	43.6	50.7	14	16.2
H-tri^{+}	12	30.4	66.0	17	20.3
H-tri^{-}	20	40.6	50.7	15	24.4
FS-di^{+}	10	30.4	50.7	10	14.3
FS-di^{-}	22	48.7	66.0	22	24.4
IgG^{+}	20	35.5	57.9	22	31.5
IgG^{-}	12	40.6	50.7	10	17.3
Prog^{+}	18	28.4	49.7	18	22.3
Prog^{-}	14	49.7	53.9	14	20.3
Estr^{+}	19	35.5	57.9	18	31.5
Estr^{-}	13	46.7	50.7	14	16.2
SAP^{+}	6	30.4	99.4	8	22.3
SAP^{-}	26	40.6	51.7	24	20.3
MBL^{+}	26	40.6	66.0	26	24.4
MBL^{-}	6	38.5	51.7	6	7.1
Con A^{+}	30	40.6	57.9	31	20.3
Con A^{-}	2	9.2	12.2	1	—

*Np = number of cases with primary breast carcinoma, features derived from the primary tumors; Nm = number of cases with intrapulmonary metastases, features derived from metastatic tumors; for further abbreviations, see footnote to Table II.

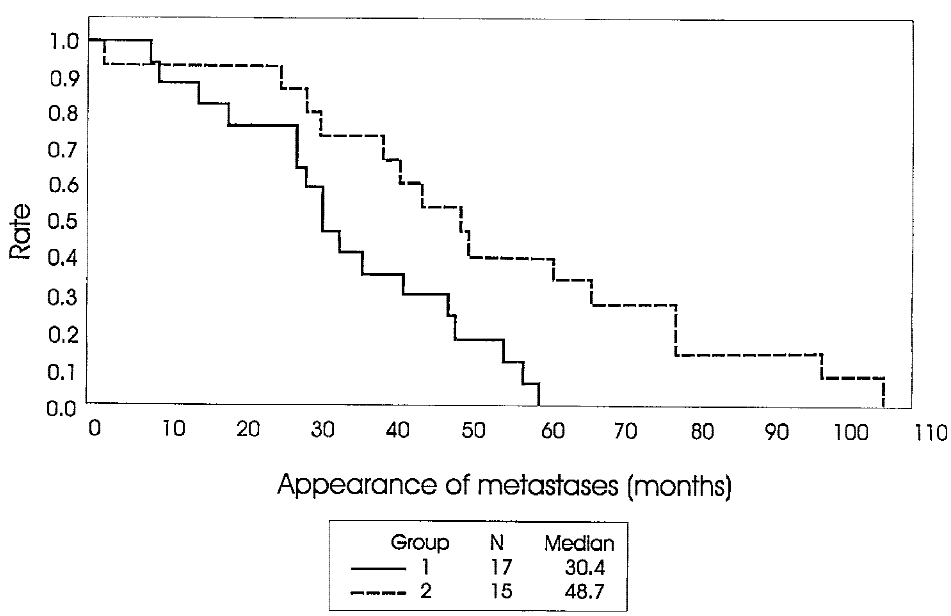

Fig. 1. Period between excision of the primary breast carcinoma and resection of lung metastases, grouped according to the median of IOD entropy (1 < median; 2 > median) ($P = 0.0101$).

after resection of lung metastases, the distance between tumor cells and lymphocytes and the expression of progesterone receptors is of significant relevance. No relationship to the clinical parameters such as pT or pN stage could be detected.

DISCUSSION

The reliable estimation of the survival of cancer patients is a continued challenge for cancer research and therapy. Nearly all women who develop breast carci-

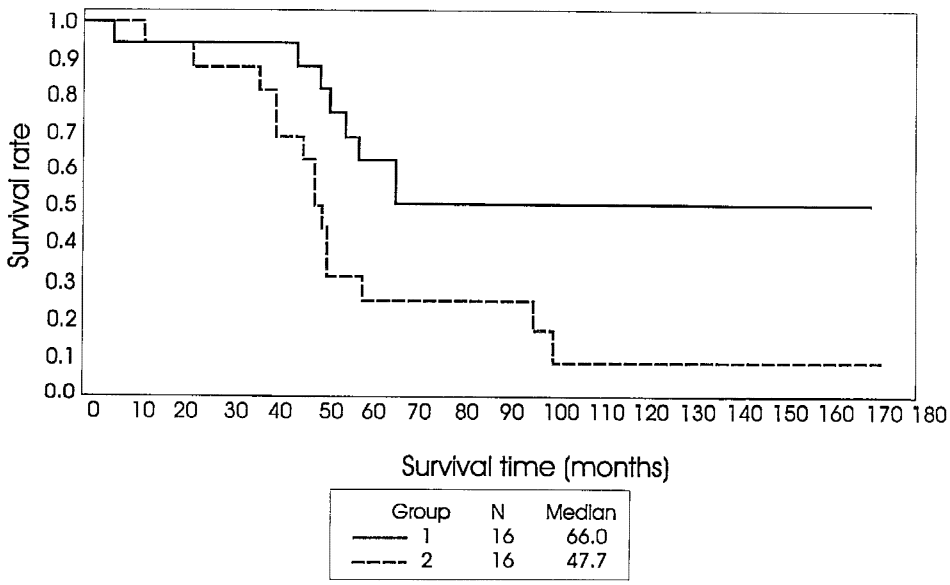

Fig. 2. Survival of patients with breast carcinoma and pulmonary metastases, grouped according to the median of distance between tumor cells and lymphocytes (1 < median; 2 > median) ($P = 0.0160$).

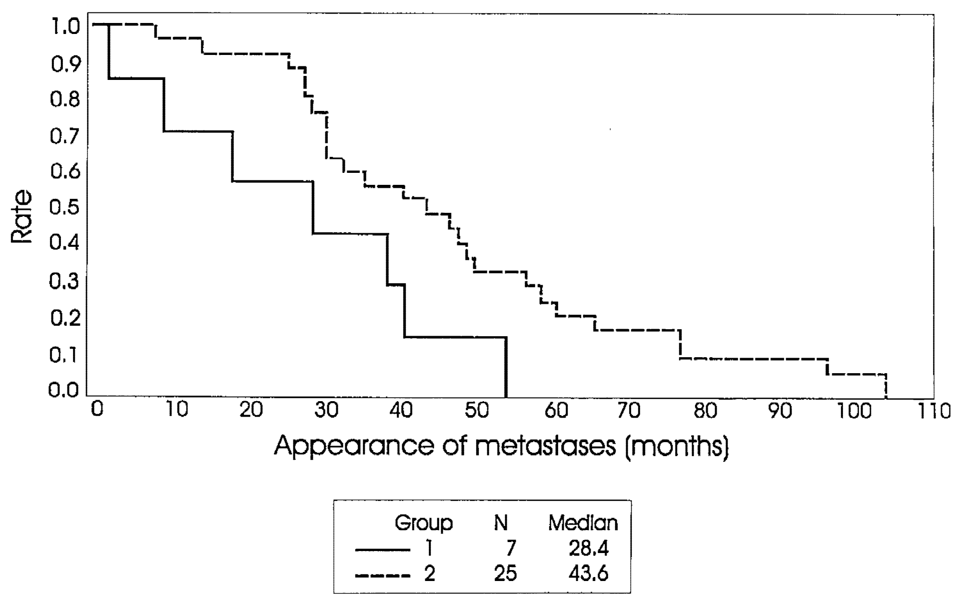

Fig. 3. Period between excision of the primary breast carcinoma and resection of lung metastases, grouped according to the expression of binding capacities for histoblood group A-trisaccharide (1 = no detectable binding sites; 2 = detectable binding sites) ($P = 0.0394$).

noma undergo surgical resection of the carcinoma, and—with consideration of the clinical stage and hormone receptor expression—adjuvant anti-hormone, cytostatic, and radiation therapy. Due to the occurrence of lung metastases besides the frequent lymph node involvement, their analysis relative to the primary tumor is of interest. The cohort of 32 patients in this study with complete data is necessarily limited due to the essential requirement to simultaneously study both types of lesion from the same patient. Therefore, statistical analysis can only be performed within this cohort in relation to the survival rates and the periods relating to the excision of lung metastases. The material was not subject to any selection bias, because no results of any primary tumor was known at the time of case collection and performance of the complete histochemical, cytometric, and syntactic structure analyses. Although only a minority of 10–20% of breast carcinoma patients develop lung metastases, their establishment still deserves analysis, as attested by this study. In view of the presented results, the following conclusions can be drawn.

A notably high percentage of breast carcinomas with limited tumor stages such as pT1, pT2, and pN0 are capable of metastasizing into the lung. The median pe-

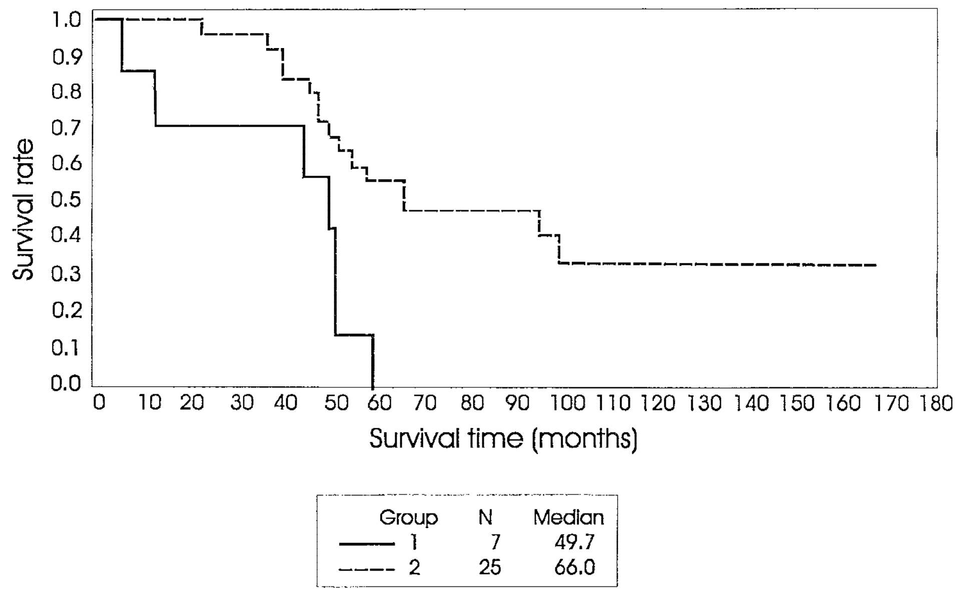

Fig. 4. Survival of patients with breast carcinoma and pulmonary metastases, grouped according to the expression of binding capacities for histoblood group A-trisaccharide (1 = no detectable binding sites; 2 = detectable binding sites) ($P < 0.0044$).

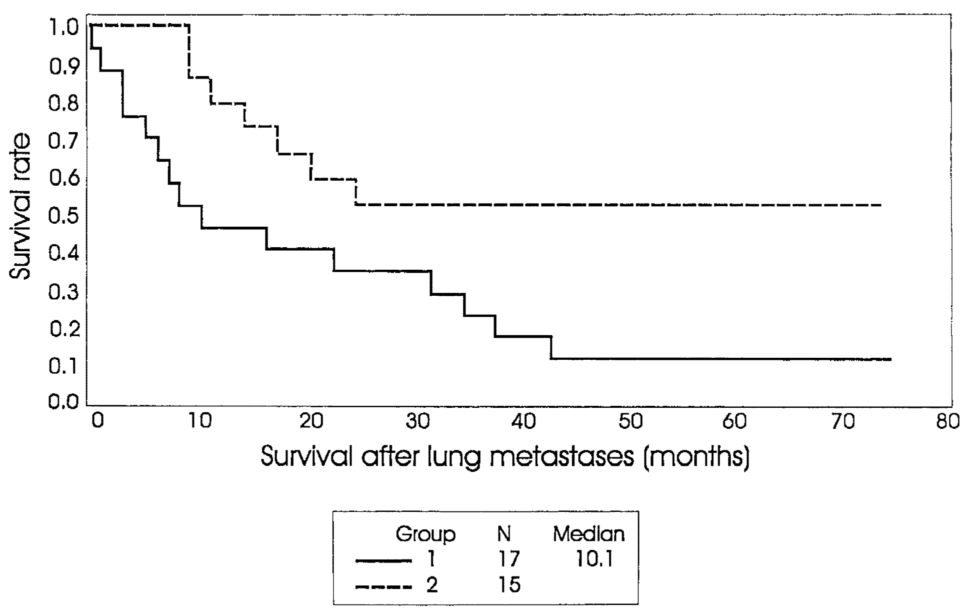

Fig. 5. Survival of patients with primary breast carcinoma and intrapulmonary metastases after resection of lung metastases, grouped according to the expression of binding capacities for histoblood group A-trisaccharide of tumor cells of intrapulmonary metastases (1 = no detectable binding sites; 2 = detectable binding sites) ($P = 0.0158$).

riod between tumor resections from the breast and lung measured 40 months. Tumor stages display—if at all—no remarkable association with the period between the diagnosis of the breast carcinoma and the development (excision) of lung metastases. Of major importance are the age of the patients, the expression of progesterone receptors as well as of histoblood group A-trisaccharide- and Forssman disaccharide-binding sites. In addition, features of the ''structural organization'' of the primary tumors (structural entropy and its current) and the spatial placement of the tumor cells with lymphocytes seem to be associated with the period to visibly establish intrapulmonary metastatic nodules. These data are in agreement with previous findings. They had revealed that exactly the same parameters are associated with the survival of patients with potentially curatively resected lung carcinoma [2,32]. The expression of binding capacities for distinct glycohistochemical probes is not independent from the cytometric and structural parameters. On the contrary, detection of binding capacities for A- and H-

TABLE V. Multivariate Analysis of Features Associated With the Period Between Excision of Breast Carcinoma and the Resection of Lung Metastasis, Overall Survival, and Survival After Resection of Lung Metastases (N = 32)*

Parameter	Rank	Wald chi-squared test
Features of breast carcinoma		
Total survival		
Age (<56, >56 years)	1	7.22
A-tri binding	2	5.79
IOD entropy	3	4.78
Progesterone receptor	4	4.23
Distance between tumor and lymphocyte	5	3.63
Period until resection of metastases		
Progesterone receptor	1	13.1
A-tri binding	2	6.47
IOD entropy	3	5.35
Age (<56, >56 years)	4	4.66
FS-di binding	5	3.16
Features of lung metastases		
Survival after resection of metastases		
Progesterone receptor	1	7.1
Distance between tumor and lymphocyte	2	6.11
FS-di binding	3	5.88
IOD entropy	4	3.34
Distance between tumors	5	2.78

*For abbreviations, see footnote to Table II.

trisaccharides is associated with a low S-phase-related tumor cell fraction and a low current of structural entropy in the primary tumor. Similar results have been reported from prostate and lung carcinoma [19,32]. One is tempted to assume that these findings reflect a basic biological meaning, i.e., a close (negative) association between tumor cell proliferation and the presence of a productive recognition system. The expression of progesterone receptors seems to be associated with a short period for development of lung metastases, whereas that of estrogen receptors has no influence at a statistically significant level.

The median survival of the women within this cohort was 59 months (about 5 years), which is long compared to the survival of patients with primary lung cancer undergoing potentially curative surgical treatment [1,2]. Prognostic features of the primary breast carcinoma include the expression of binding capacities for histoblood group A-trisaccharide, the expression of progesterone receptors, the IOD entropy, and the distance between tumor cells and lymphocytes. Although no clear-cut association between the expression of the binding capacities for the probes between the primary breast carcinoma and their intrapulmonary metastases could be observed, the features of the metastases appear to exert an additional influence for the period after metastases resection and death. The estrogen receptor status, which is an important factor for therapy selection, is not associated with the length of survival. In addition, the tumor stages are of no importance in calculating the survival of patients within this cohort.

The overall survival after diagnosis comprises the period between excision of the primary breast carcinoma and the development of intrapulmonary metastases, and the length of survival after the resection of the metastases. The median survival of the patients after resection of the lung metastases was 20 months. None of the determined features measured in the primary breast carcinoma is associated with the length of this period at a statistically significant level. Cellular parameters in the metastases relevant for survival after metastasis excision include the presence or lack of binding capacities for the histoblood group A-trisaccharide and for the Forssman disaccharide, the IOD entropy, and the distance between tumor cells and lymphocytes. The opposite trend of the binding reactions with the two neoglycoconjugates which share the α1,3-GalNAc terminus is definitive evidence for the inherent specificity and the relevance of the glycocode [14,15,22,32]. This result, too, attests to the necessity to include the expertise of carbohydrate chemistry into optimal marker design. In addition, the estrogen receptor status is associated with this survival period. Apparently, the estrogen receptor status is an indicator for improved survival at this stage, but not for the risk of development of intrapulmonary metastatic growth. The intrapulmonary tumors appear to behave as an independent biological system, not merely reflecting the feature of the entire primary tumor. In line with a wealth of information on selection of subpopulations and the impact of microenvironmental factors, which have a bearing on glycohistochemical parameters [39], there are presumably different effector mechanisms. Concerning survival, the assessment of the features of the metastatic lesions surpasses the relevance of those of the primary tumors.

This study supports the notion that the monitoring of expression of binding sites for innovative probes, i.e., synthetic neoglycoconjugates, is at least phenomenologically linked to features measured by image analyzing systems, i.e., morphometric features of the tumor cell nuclei and the structural organization of the tumor cell compartment. The structural entropy which describes the energy balance and growth efficiency of a biological system embedded in its host organ deserves further attention [32]. It is not surprising that these properties are powerful descriptors of a thermodynamically open system. Its growth velocity is not only related to the energy supply, but also to the efficiency of its growth mechanisms [33,38]. Simple features such as the minimum distance between neighboring tumor cells or the calculated entropy are also correlated with survival in the analyzed cohort. Thus, we underscore the proposed relevance to systemically extend the

measurement of these parameters in a combined setting [32].

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